Protocols for SeaBASS
November 2013

CELL COUNTS

The coccolith and coccolithophore enumeration technique involves filtering 50-100 mL of seawater sample onto a 25 mm, 0.45 um pore size Millipore HA filter. Filters are rinsed with borate buffer and are placed in a petri plate and immediately frozen at −20°C for 12 hours then dried in a drying oven at 60°C for 24 hours. Filters are mounted onto glass slides using Norland Optical Adhesive 74 which is cured under UV light (previously Canada Balsam was used instead of optical adhesive).
On shore, the coccoliths are viewed in a polarizing microscope where they show up as bright particles against a dark field. Slides are automatically imaged using an Olympus BH2 microscope (SN 222441) equipped with a 40X objective and 10X ocular attached to a QImaging-QuickCam Fast 1394 cooled monochrome, 12 bit digital camera (SN32-0090B-316). Image acquisition software controls the stage and focus. One hundred and fifty to two hundred images of each filter are taken and each image is viewed in order to verify focus and eliminate any poorly focused images. Enumeration of detached coccoliths and plated coccolithophores is performed by the analysis software “CCC.”

References:

Balch, W. M., D. T. Drapeau, B. C. Bowler, E. Lyczskowski, E. S. Booth, and D. Alley. 2011. The contribution of coccolithophores to the optical and inorganic carbon budgets during the Southern Ocean Gas Exchange Experiment: New evidence in support of the “Great Calcite Belt” hypothesis. Journal of Geophysical Research – Special Issue 116, C00F06: pp. 1-14.

Haidar, A.T., H.R. Thierstein. 2001. Coccolithophore dynamics off Bermuda (N. Atlantic). Deep-Sea Research (Part II, Topical Studies in Oceanography). Vol. 48, no. 8-9, pp. 1925-1956.

BIOGENIC SILICAS

To determine reactive silicate, 200 mL of seawater sample is filtered onto a 25 mm, 0.4um pore size polycarbonate filter. Filters are folded and placed in a super clear polypropylene centrifuge tube and dried in a drying oven at 60°C for 24 hours then tightly capped and stored until analysis. On shore, 0.2N NaOH is added and the sample is placed in a 95C water bath. The digestions are then cooled and neutralized with 1N HCl. After centrifuging, the supernatant is transferred to a new tube and diluted with MilliQ water. Molybdate reagent is added and then a reducing agent is added to reduce silicomolybdate to silicomolybdous acid. The transmission at 810 nm is read on a Hitachi U-3010 spectrophotometer (SN 0947-010). Reactive silicate is calculated using a silicate standard solution standard curve prepared at least every 5 days or whenever new reagents are prepared. Readings are corrected using a reagent blank run at the same time as the standard curve and three tube blanks interspersed in each batch.

References: Brzezinski, M.A. and D.M. Nelson. 1989. Seasonal changes in the silicon cycle within a 618 Gulf Stream warm-core ring. Deep -Sea Research I no. 36, pp. 1009-1030.

JGOFS. 1994. Joint Global Ocean Flux Study Core Measurement Protocols, JGOFS Report #6. SCOR, Halifax, N.S., Canada, pp. 107-110 (Chapter 12: The Determination of Reactive Silicate in Sea Water)

Strickland, J.D.H. and T.R. Parsons. 1977. A practical handbook of seawater analysis. Fisheries Research Board of Canada Bulletin 167, pp. 65-70.

PIC (Particulate Organic Carbon)

Water samples are filtered through a 25mm, 0.4 µm pore size polycarbonate filter. The dry filter is rinsed with Potassium tetraborate (6.11 g/l $K_2B_4O_7 \cdot 4H_2O$) buffer while still in the filter tower to remove as much seawater salt and also to maintain a high pH (~8.1) during sample storage and preserve the $CaCO_3$ on the filter. Filters are placed into trace metal clean polypropylene centrifuge tubes and dried at approximately 60°.

For analysis, the filters are currently sent out to the Sawyer Environmental Chemistry Laboratory at the University of Maine or Department of Earth Sciences at Boston University. Filters are digested in a 5% nitric acid solution for 12 hours to dissolve all $CaCO_3$ and the solution is analyzed by ICP-AES (Inductively Couple Plasma – Atomic Emission Spectrometry) for Ca concentration. We have filter and dissolution blanks as well as QC standards run with each batch of samples. We also use the concentration of dissolved Na in the digestate to correct for any Ca present in sea salts left on the filter. PIC concentrations are calculated using the volumes of water filtered and the volume of the digestions, and assuming all Particulate Inorganic Carbon is in the form of $CaCO_3$.

History:
These analyses go back to 2007 (GOM) and AMT 18, GasEx, and COPAS. Earlier samples were sent to Scripps UCSD (GOM 1998-2006, EqPAC 2004,2005) and AMTs 15-17 were analyzed at the National Oceanography Centre, Southampton UK.

POC (Particulate Organic Carbon)

Water samples are filtered onto 25mm GF/F filters which have been pre-combusted (450°, 5 hours). Filters are rinsed with filtered seawater (FSW) and then stored in individual petri-plates and dried (60°) for storage. Prior to analysis, the plates are opened and placed overnight in a sealed container like a dessicator with saturated HCL fumes to remove any PIC. We send these samples to the University of Maine's Darling Marine Center for analysis. The filters are packed into pre-combusted nickel sleeves and analyzed on a Perkin Elmer 2400 Series II CHNS/O for C, N, and H.

The analyzer is calibrated using tin capsules as blanks and acetanilide to calibrate instrument response to carbon and nitrogen. NIST certified check standards consisting of either low organic

content soil or sediment are analyzed to determine accuracy of carbon detection. NIST certified organic check standards such as corn flour or rice flour are analyzed to determine the accuracy of nitrogen detection. If values vary by more than 4% from stated values, instrument is examined, any problems are addressed and instrument is recalibrated and check standards rerun until error is within acceptable limits. Duplicate samples are run during each sample run to ensure results are reproducible. If duplicates cannot be run on actual samples, as in the case of filter samples, duplicate check standards are analyzed. Duplicate samples typically vary less than 2%.
One instrument blank is analyzed for every 12 samples run. One acetanilide standard is analyzed for every 15 samples run. If blank or acetanilide values differ significantly from previous values, a new series of standards and blanks are analyzed to recalibrate the instrument.
The actual minimum detection limit (3 times the standard error) determined from the standard error of the instrument blanks is 2 micrograms for carbon and 4 micrograms for nitrogen.

History:

These analyses began at UMaine in 2009 (GOM and AMT18). Previous samples were sent to the Marine Sciences Institute, UCSB (1998-2008) and processed in a similar manner. AMTs 15-17 were analyzed at the National Oceanography Centre, Southampton UK.

References:

JGOFS. 1994. Joint Global Ocean Flux Study Core Measurement Protocols, JGOFS Report #6. SCOR, Halifax, N.S., Canada, pp. 123-125 (Chapter 15. Determination of Particulate Organic Carbon and Particulate Nitrogen)

NUTRIENTS:

Water samples are collected in clean 60ml plastic bottles and immediately frozen (-20°). These samples are kept frozen with dry ice and sent to the University of California, Santa Barbara's Marine Science Analytical Lab. They are analyzed on a Lachat QuickChem 8000 for Nitrite, Nitrate plus Nitrate, Phosphate, and Silcate.

Chlorophyll a

Water samples are filtered onto a 25mm Millipore HA filter (mixed cellulose ester, 0.45 µm pore size). The filters are transferred to test tubes filled with chilled 90% acetone for extraction and vortexed until the filter dissolves. Tubes are stored in the dark in a freezer for 24 hours before analysis. Tubes are then re-vortexed and gently centrifuged (~1300g) for 5 minutes before being decanted into a glass cuvette for the fluorometer. We use a Turner Designs 10AU to read F_b of the sample and then add 50 µl of 10% HCL and read F_a. The fluorometer was calibrated previously with a pure chlorophyll extract (Turner Designs part# 10-850) to determine Tau $\tau=(F_b/F_a$ pure chl a) and chlorophyll a can then be calculated from: $(Fb - Fa) * (\tau/ \tau-1) * (V_{filtered}/V_{extracted})$. Generally all surface measurements are made in triplicate.

References:

Trees et al., Fluorometric Chlorophyll a: Sampling, Laboratory Methods, and Data Analysis Protocols. Chapter 3. Ocean Optics Protocols for Satellite Ocean Color Sensor Validation, Revision 5, Volume 5.